# European Friedreich's Ataxia Consortium for Translational Studies

## Reporting

### Project Information

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**Final Report Summary - EFACS (European Friedreich's Ataxia Consortium for Translational Studies)**
Executive Summary:
EFACTS (the European Friedreich’s Ataxia Consortium for Translational Studies) assembles a body of expertise to adopt a translational research strategy for the rare autosomal recessive neurological disease, Friedreich ataxia (FRDA).

FRDA is a severely debilitating disease that leads to loss of the ability to walk and dependency for all activities. Some patients have cardiomyopathy that can cause premature death, visual and auditory loss, kyphoscoliosis, pes cavus, and diabetes. Onset is usually in childhood, but it may vary from infancy to adulthood. FRDA involves child health and ageing aspects.

FRDA affected individuals and clinical specialists are dispersed. This is a hindrance for patients to receive the care they need, and for clinicians and researchers to make progress. EFACTS has been created to move past this limitation.

The consortium, led by Professor Massimo Pandolfo (Neurologist, Université Libre de Bruxelles, Brussels, Belgium) acting as coordinator, possesses expertise ranging from clinical neurology, biochemistry, structural biology, systems biology, genetics and epigenetics. These 14 clinical and basic investigators are undisputed leaders in FRDA research and have provided major contributions to the current knowledge of this disease. EFACTS strongly believes that, 12 years after European researchers discovered the FRDA gene, frataxin, when new treatments for FRDA are being developed, the time is ripe to invest in FRDA research in a concerted Europe-wide fashion.

EFACTS gathered the critical mass of researchers and clinicians to exploit the patient base, research reagents and knowledge for progress. This came when IT can act as a crucial support for collaborative work in collecting patient data and material, making it available to leading researchers for advanced analysis, research and drug development.

To this end, this project has drawn up and executed a joint programme of research with the following scientific and technological objectives, providing a model for translational European research targeted to a specific rare disease, which can ultimately improve the patients’ health and quality of life.

1. Comprehensively populate a European FRDA database, linked to a bio bank
2. Define a panel of clinical assessment tools that can be used to define outcomes for clinical trials and identify the multiple health problems that occur during the course of FRDA disease progression
3. Build on the knowledge base of frataxin structure and function
4. Build on the knowledge base of frataxin-related cellular homeostasis and the pathogenic cascade
5. Build on the knowledge base of epigenetic mechanisms regulating frataxin silencing
6. Develop new and improved cellular and animal models for the study of FRDA
7. Identify novel FRDA biomarkers
8. Identify genetic modifiers of FRDA disease
9. Develop novel therapeutic strategies for treating FRDA

Project Context and Objectives:

Concept
The overall concept developed by EFACTS (the European Friedreich Ataxia Consortium for Translational Studies) is to assemble a body of expertise that can adopt a fully translational research strategy applied to the study and treatment of Friedreich ataxia (FRDA), a rare monogenic neurological disease. FRDA is a severely debilitating disease that leads to loss of the ability to walk and dependency for all activities. Some patients have cardiomyopathy that can cause premature death, visual and auditory loss, kyphoscoliosis,
Pes cavus, and diabetes. Onset is usually in childhood, but it may vary from infancy to adulthood. FRDA affected individuals and clinical specialists are dispersed. This is a hindrance for patients to receive the care they need, and for clinicians and researchers to make progress. EFACTS has been created to move past this limitation. The consortium possesses expertise ranging from clinical neurology, biochemistry, structural biology, systems biology, genetics and epigenetics. These clinical and basic investigators are undisputed leaders in FRDA research and have provided major contributions to the current knowledge of this disease.

Objectives
EFACTS overall goal is to progress our understanding of FRDA through basic research and harness this for identifying novel disease-specific biomarkers and drug therapeutics. This will be coupled to improving the methods of clinical assessment and diagnosis, and the implementation of the first pan-European FRDA database registry, linked to bio banks of patient material.

The totality of objectives provide a model for translational European research targeted to a specific disease, which can ultimately improve the patients' health and quality of life.

To this end, this project has drawn up a joint programme of research with the following scientific and technological objectives:

1. Comprehensively populate a European FRDA database, linked to a bio bank
2. Define a panel of clinical assessment tools that can be used to define outcomes for clinical trials and identify the multiple health problems that occur during the course of FRDA disease progression
3. Build on the knowledge base of frataxin structure and function
4. Build on the knowledge base of frataxin-related cellular homeostasis and the pathogenic cascade
5. Build on the knowledge base of epigenetic mechanisms regulating frataxin silencing
6. Develop new and improved cellular and animal models for the study of FRDA
7. Identify novel FRDA biomarkers
8. Identify genetic modifiers of FRDA disease
9. Develop novel therapeutic strategies for treating FRDA

Objectives 1-2
An IT-based patient registry, fulfilling all data protection requirements, has been implemented. Up to 30th April 2015, 604 patients were enrolled, forming a “core” cohort with a planned minimal follow-up of two years. So far, close to 83% have undergone their first annual follow-up assessment, and 71% already came back for the second one. Baseline data of the “core” cohort have been analysed and published in Lancet Neurology. A biological repository has been established, where samples from EFACTS patients are stored for analyses. It currently contains 559 baseline and 461 1 year follow-up samples.

Objectives 3-4
EFACTS beneficiaries could establish that frataxin, by controlling both iron entry and sulfide production, is essential to properly assemble and protect the Fe-S cluster during the initial stage of biogenesis. They confirmed that in vitro human frataxin functions as an enzyme activator while, under the same conditions, the E. coli homologue CyaY is an inhibitor of Fe-S cluster biosynthesis. Conversely, the previously postulated participation of frataxin in heme biosynthesis could not be confirmed. Investigations on the pathogenic cascade in FRDA included analyses of mitochondrial function, iron metabolism, ROS production, apoptosis and signalling pathways in human and mouse cells, and a proteomic study in a fly model.
Objective 5
Epigenotype analysis has identified key histone and DNA modifications associated with FXN gene silencing triggered by expanded GAA repeats. A novel methodology enabling the localisation of the FXN gene and its activity to specific regions in the nucleus in living cells has been established – this is important for understanding the mechanisms underlying the silencing of the gene in FRDA and provides an excellent system for studying the ability of novel therapies to relocate genes from silencing compartments to active regions in the nucleus. Additionally, a novel rapid technique for assessing DNA methylation, allowing confirmation of specific DNA methylation sites in FRDA, has been developed.

Objective 6
Construction of a complete human transgene with large GAA repeat expansion flanked by loxP sites and with a mutated pausing site is under way. It will be used to generate a mouse model closely replicating the genetic and epigenetic features of the human disease. The development of cellular models has focused on the use of induced pluripotent stem cells (iPSCs). EFACTS published data show for the first time that iPSCs and their neuronal and cardiac derivatives allow to study mitochondrial damages and GAA expansion instability in FRDA.

Objective 7
Collection of biological samples for biomarker studies is ongoing. A first study on gene expression profiles in FRDA PBMCs established a core biomarker set and evaluated how it is affected by compounds that restore frataxin expression (HDAC inhibitors). A subsequent study showed that ex vivo nicotinamide treatment of PBMCs normalizes about 67% of these biomarkers, confirming that they can be used to monitor response to pharmacologically induced increase of frataxin levels.

Objective 8
The identification of genes that modify the frataxin deficiency phenotype or affect frataxin silencing by expanded GAA repeats has been pursued using the powerful genetic approaches that are possible in Drosophila. New lines carrying expanded GAA repeats were screened for modifiers of GAA repeat-induced silencing and for modifiers of repeat stability. Along with bioinformatics analysis of biomarkers obtained from all relevant beneficiaries and from published data, these studies will yield important clues about FXN gene silencing and the downstream effects of FXN silencing.

Objective 9
EFACTS has focused on potential therapeutics to increase FXN expression, with these main achievements so far:
- The first-in-human study of diphenylamide HDAC inhibitors has shown that these molecules can safely up-regulate frataxin expression in FRDA patients, at least in peripheral tissues.
- The identification of a new compound, C5 that could be a potential therapeutic for FRDA.
- Three compounds (IFN-γ, NAM, and a proteasome inhibitor) have been tested in the YG8 mouse model of FRDA with encouraging results.
- The proof-of-concept demonstration in an animal model that AAVrh10-based gene therapy can restore frataxin expression in the hearth and reverse FRDA cardiomyopathy even when already symptomatic.
- Phase 1 clinical testing of nicotinamide, an HDAC class 3 inhibitor, with positive results in terms of safety and FXN induction.
- Phase 1 clinical testing of a 2-aminobenzamide HDAC inhibitor, with positive results in terms of safety and FXN induction.

Project Results:
WP1&2 had the following objectives: 1) to establish a European IT-based patient registry / database, 2) to design, implement and populate an integrated clinical and basic science database, 3) to follow and quantify disease progression according to sensitive and robust clinical, activities of daily living (ADL), quality of life (QOL), and functional measures 4) to analyse both clinical and basic science data in order to identify novel biomarkers and genetic modifiers - aid prognosis prediction - better understand the molecular biology and biochemistry of the disease - assist in the development of novel therapies, 5) to investigate cognitive function in Friedreich ataxia, 6) to use MR imaging and PET of the brain to study disease pathogenesis and disease progression, 7) to develop a user-friendly software utility to enable access to and analysis of clinical and basic science data for all Beneficiaries and 8) to obtain, and establish a repository of, biological samples for studies on pathogenesis, generation of cell models, and biomarker development.

Beneficiary 2 has subcontracted 2mt software GmbH (Ulm, Germany) for the setup and maintenance of an IT-based platform that fulfils all data protection requirements. The structure and plausibility checks for the database were designed and implemented. The platform has a web-based electronic data capturing and export system. It is possible to export data to and interface with the analysis tools developed by Beneficiary 9b. 2mt software GmbH has continued to expand the platform with additional eCRFs (i.e. for collection of cognitive data, quality-of-life data, sub studies), dealt with technical user enquiries, implemented improvements in the data monitoring functions and data export functions, and managed user accounts.

Up to 30.4.2013 2 years after the inception of the registry, 604 patients were enrolled in the database. Seven out of the 11 clinical centres have exceeded their initial individual recruitment goals. The network has agreed on including new clinical centres led by PIs known to be experts in ataxia research, if they approach the network with the wish to join.

The clinical network has been following the 604 patients (core sample) for two years after their inclusion date and each patient's data is updated annually. After the conclusion of funding through the EC, the registry will continue to run with an open cohort to recruit the maximum number of patients. Since 1.5.2013 an additional 49 new patients have been included in the database by six different centres, which has helped to buffer attrition rate of the core sample due to death, loss of contact, unavailability for assessments due to the severity of the disease, and withdrawal from the study (39 patients). According to database entries on 30.4.2015 83% of the core sample (501 patients) returned for their first annual follow-up assessment. This was accomplished thanks to the extension granted to EFACS project. There was a loss to follow-up at the 1-year due to the clinical centre in Madrid losing study staff during the 1-year follow-up window. Seventy-one percent of core sample patients (426 patients) have returned for their second annual follow-up assessment. Follow-up assessments should always take place 1-year +/- 1 month from the previous assessment date; thus completion of 2-year follow-up entries was planned for 31.5.2015. One hundred and twenty-six patients (21%) have already returned for a 3-year follow-up assessment in seven of the 11 clinical centres. Two of the clinical centres have also begun to see patients for their fourth annual assessment.

Baseline data of the core sample were published in The Lancet Neurology (Reetz et al.). The Scale for the Assessment and Rating of Ataxia (SARA) and the Inventory of Non-Ataxia Signs (INAS), the performance-based coordination test Spinocerebellar Ataxia Functional Index (SCAFI), the neurocognitive phonemic
verbal fluency test, the activities of daily living (ADL) part of the Friedreich Ataxia Rating Scale (FARS) and EQ-5D were used as primary outcome measures. The Friedreich ataxia cohort (592 patients) was subdivided into three groups: early disease onset (≤14 years), intermediate onset (15-24 years), and late onset (≥25 years), which were compared for clinical characteristics and outcome measures. Age of disease onset was inversely correlated with the number of GAA repeats in the frataxin (FXN) gene: every 100 GAA repeats on the smaller repeat allele were associated with a 2.3 year earlier onset. There was a significant estimated annual worsening of SARA, INAS, SCAFI, verbal fluency, and ADL during the first 25 years of disease. For SARA, the predicted annual rate of worsening was significantly higher in early-onset patients and intermediate-onset patients than in late-onset patients. This study is registered with ClinicalTrials.gov number NCT02069509.

Three different clinical centres proposed four sub-studies and measures have been incorporated into the database.

Beneficiary 14 has been testing a novel device (Opynov SAS, Grenoble, France) examining dysmetria and manual control. Data from this sub-study were published in Neurology (Filipovic Pierucci et al., 2015).

Based on the analysis of 179 patients with Friedreich ataxia through the EFACTS (European Friedreich Ataxia Consortium for Translational Studies) network, 77 patients with spinocerebellar ataxia, 48 adult controls, and 120 healthy children we have demonstrated that the electronic CCFS is a quantified measurement of cerebellar ataxia independent of age, usable in individuals aged from 7 to 80 years. The automated nature of the electronic test device makes it reproducible between operators and centres, as well as easy to use.

Beneficiary 6 aimed to quantitatively assess hand-motor skills with a game-software incorporated in a hand-held device, such as an iPhone or iPod. 153 data sets of patients and controls including follow up data were collected at two study sites.

Beneficiary 8 has proposed two sub-studies looking at a) patients’ self-reported family history of diabetes and b) malignancy in the patient. These are based on the fact that FRDA patients commonly suffer from type 1 diabetes with the pancreas being a typical non-neurological site of morbidity and the assumption that FRDA patients might be predisposed to cancer as indicated by animal studies.

Monitored data can be exported at any time in well-known formats such as CSV, EXCEL, SAS, CDISC. Online reports can be defined for descriptive evaluations. After each year, data have been routinely exported and made available for analysis. Partners can download their specific data at any time. Groups with existing sub-studies have been receiving data for analyses as requested and all participating centres can propose exports of data after approval of additional sub-studies by the steering committee. Data from 1-year follow-up assessments are closed. Currently, we are finalizing 2-year follow-up data entry and monitoring, so that data will be available for analysis by end of 2015.

A database for nonclinical data has been created and populated. The database is searchable and the data available for download by beneficiaries. We have continued to populate the database with nonclinical data throughout the project and have deposited datasets consisting of images, transcriptome, proteome, CHIP seq both baseline and time series data, from various tissues both of mouse and drosophila models as well as human subjects.

We have developed software tools for analysing various aspects of the available data (in the consortium or in public databases). Some of these tools are available online and can be accessed and used in a web based user friendly manner while others have to be installed and run on users’ machines. These tools and their use in supporting the various activities of the consortium are outlined below:

RNA seq analysis software
Bespoke RNA seq data analysis software has been developed to study RNA seq data from both human drug trials and mouse model analysis. The analysis has resulted in a publication (Chan et al.)

Transcriptomics Meta-analysis Methodology
Methodology for the comparison of gene expression data from both the various mouse models as well as human studies has been developed to compare and contrast the results from these diverse studies. This is in order to identify common disease pathways and genes that are important in FRDA. More specifically microarray data from partners within the consortium (Beneficiaries 1, 9a and 13) and from researchers in the wider community have been analysed using meta-analysis techniques to identify common features in the data across the currently available mouse models and to compare the mouse models with human data. Additional time course data from two of the mouse models have been analysed to determine the genes involved in the early stages and the development of FRDA. The tool we have designed has a GUI supporting display of the graphics and statistical results, and enables comparisons to be made across data samples. The tool is written in C++ and is available within the consortium.

Web based motif finding in multi-relational networks
In order to identify interactions in biochemical and gene regulation networks we have developed a web-based easy to use bioinformatics tool able to identify motifs in multi-relational networks (Comsa et al.). The tool allows us to identify interactions between genes or proteins and hub proteins or genes from either proteomic or transcriptomic data. In this way we can identify pivotal genes and interactions in the animal models or human samples. We have used this tool for network comparison of the differentially expressed genes in various tissues in the mouse models available within the consortium. The results suggest that the different mouse models display different aberrant gene profiles dependant on the tissue type examined and the model. The models are very different in the aspects of the human disease phenotype that they display. These different models can be regarded as intrinsically useful however no one model can be employed to test and observe all the different facets of the human disease. This result is supported by data from phenotypic studies of the mice which demonstrate the very dissimilar phenotypes displayed by the different models.

Comparison with human patient data has shown that the genes that are aberrantly expressed in common with the mouse models are in the minority; however this could be due to the different tissue tested in patients compared with the mice. Data from different studies of aberrant gene expression in patients from various sources have been analysed for common genes, ontologies and affected pathways. Data from juvenile patients has been analysed to try to identify genes that are involved in disease progression.

DNA Methylation Analysis
A software package has been developed to assist in identifying DNA motifs associated with aberrant methylation in DNA using association rule mining techniques. We examined sequences surrounding both the variably methylated (VM) CpGs, which are hypermethylated in patients compared with unaffected controls, and the non-variably methylated CpGs which remain either always methylated (AM) or never methylated (NM) in both patients and controls. Using the J48 algorithm of WEKA analysis we identified that two patterns are all that is necessary to classify our three regions. AM can be distinguished from VM by the sequence CCGG* which is found in VM and not AM regions. There are no unique patterns for the NM region however we observed that AATT* distinguished between NM and VM + AM using proportional frequency. Thus we have evidence that the DNA sequence surrounding a CpG can influence its susceptibility to be de novo methylated in a disease state associated with a trinucleotide repeat. This work has been published (Ghorbani et al. 2013).

Intelligent Data Analysis of Clinical Data

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The clinical data has been analysed using computational data analysis techniques to attempt to identify which features are the most informative in classifying the patients into subgroups, identifying key stages in the disease progression and predict disease progression in patient subgroups. Hill Climbing followed by the J48 algorithm has been used to identify subgroups of patients that differ in their clinical attributes. The strongest classifier of age of onset disease subgroups is the GAA repeat number since any analysis removing this feature decreased the value of kappa (a measure of the accuracy of classification). This indicates the importance of accurate measurement of the number of repeats as a prognosis tool. The features that were important as markers of disease progression were identified by classifying which features were most informative at particular time points after the onset of the disease (disease duration). These were elements of the SARA score, cardiac and activities of daily living, placing less importance on INAS. The informative features differed according to the age of onset showing that the disease can be divided into sub types according to onset age. Interestingly cardiac features are not informative as indicators in patients with an age of onset above 26 years indicating that these features do not change significantly with time in these patients. The converse was true for patients with an onset of 5 years or younger. Our results indicated a possible difference in how the disease affects males and females.

Through our analysis we can show that cardiac features are less severe in females than males.

Modelling the MAPK signal transduction pathway

There are several computational models of this pathway which allow running simulations with different kinetic rates and different levels of concentrations for the proteins of the pathway. We employed the Hornberg 2005 model and encoded it in Matlab using a system of ordinary differential equations (ODEs). The gene expression levels of wild-type mice and the MCK mouse model have been measured at different time points using microarray methodology and used to model the pathway.

We have designed a software utility to manage the database and allow data to be run through analytical routines. The data in both the non-clinical database and clinical databases is easily found and downloaded using the GUI interface designed to allow for ease of use by all partners. The data is searchable using many terms e.g. data type, depositing lab, date of entry and more. The data can then be downloaded in formats e.g. .csv files which allow easy analysis by the software and methodologies outlined above and as yet unutilised ones in the future. The password-protected utility provides an easy to use gateway for all partners to have privileged access to unpublished data. The nonclinical database is a MySQL database, accessible with any operating system. The user interface has been developed in a friendly way and can accept any type of files: .zip files, images (of any kind), text files, .csv and .xls files, etc.

All EFACTS clinical Partners have been contributing biological samples from FRDA patients, including cells, DNA, RNA, blood and urine, to be utilised for studies on pathogenesis (WP4), epigenetics (WP5), generation of cell models (WP6), biomarker development (WP7), and studies of modifiers (WP8). A general repository for blood and urine samples is located at the site of Beneficiary 2. Each biological sample is being inventoried in the EFACTS IT platform, where samples are linked to relevant demographic, clinical, biological and genetic data. As of 30.4.2015 559 baseline visit samples and 461 1-year follow-up visit samples (including core sample patients, new patients, and controls) are stored in and can be selectively retrieved from the repository.

The combination of genetics, functional and cognitive data, and biomaterial samples in the EFACTS registry provides a broad database for future studies and reliable predictors of disease progression, moving us closer to identifying outcome measures and target points for therapeutic trials in this devastating multisystem rare disease.

Furthermore, neuropsychological assessments in 23 FRDA patients showed deficits in social cognition,
information processing, and verbal fluency when compared to a group of age-, gender and education-matched healthy controls. Analysis of structural and functional magnetic resonance imaging (MRI) data, acquired in 16 patients and matched controls, is almost completed. In addition to cerebellar volume loss in FRDA, particularly in lobule VI, we found differences in functional activity while processing a verbal fluency task. Patients showed lower neural response in the left cingulate cortex and orbitofrontal cortex for phonemic verbal fluency, and in right Area 44, left parietal and temporal regions for the semantic condition. During the phonemic condition patients also exhibited enhanced activity compared to controls in left Area 44, anterior insula, prefrontal cortex, as well as in the right cerebellum (lobule VI and Crus I), parietal and occipital areas. This pattern of neural response underlying verbal fluency deficits suggests disruptions in cerebro-cerebellar pathways in FRDA with compensatory recruitment of fronto-insular regions that may be related to posterior cerebellar damage. These findings are being presented at the national conference “Deutsche Gesellschaft für Neurologie” (DGN) in September 2015. In order to further evaluate our interpretation of results we are currently performing subsequent analyses to unravel alterations in structural (via diffusion-tensor imaging) as well as functional connectivity in FRDA. This will help us to gain a better understanding of the cerebro-cerebellar mechanisms underlying the activity differences found in the fMRI task. Finally, follow-up measurements using the same MRI protocol and additionally including examination of the spine are ongoing and will be used to assess longitudinal structural and functional changes in FRDA as well as spinal anomalies.

All these clinical results will serve for therapeutic trial planning, but they will also be used, along with the sub-studies described, to identify potentially modifiable factors, and therefore areas of intervention, affecting disease progression and impact on patients’ lives.

WP3: Study of Frataxin function

WP3 is dedicated to understanding the function of frataxin in the enzymatic reaction involving iron homeostasis using solid in vitro biochemical approaches directly validated with confirmatory in vivo approaches. In particular, EFACTS planned to establish the role of frataxin in Fe-S cluster and heme biosynthesis, and to determine the structural interactome of frataxin.

EFACTS has identified new competing interactions that modulate the role of frataxin and furthered in characterizing the frataxin interactome. In this same context, EFACTS made substantial progresses in characterizing the human iron-sulfur cluster (ISC) biogenesis machine. Beneficiary 10 has developed an efficient bacterial expression system for the production of all components of the bacterial iron sulfur cluster (isc) operon that could allow structural, functional and enzymatic studies. Mammalian frataxin has been proposed to be a multifunctional protein based on its numerous reported interactions: components of the ISC biosynthesis machinery, ferrochelatase, mitochondrial aconitase, succinate dehydrogenase, and several chaperones. To obtain a comprehensive insight into the postulated multiple functions of mammalian frataxin, Beneficiary 13 has identified and characterized the interactions of human frataxin by combining in vitro and in vivo approaches. Through immunoprecipitation experiments, Beneficiary 13 has shown that the main endogenous interactors of a recombinant human frataxin are ISCU, NFS1 and ISD11, all components of a multiprotein complex localized in the mitochondrial matrix that assembles ISCs. NFS1 is the functional cysteine desulfurase that provides the sulfur for ISC formation, while ISCU is a scaffold protein on which the newly formed ISC is built. The two proteins interact with each other in the presence of ISD11 to form the core ISCU/NFS1/ISD11 complex.
involved in de novo ISC biogenesis. Using a heterologous expression system, Beneficiary 13 has demonstrated that mammalian frataxin interacts with the preformed core complex, rather than with the individual components. These studies confirm a role of frataxin in ISC biogenesis, but do not support other postulated functions.

Beneficiary 10 has structurally characterized most ISC components in isolation and in relation to each other (Prischi et al., 2010) and carried out a study in which enzyme kinetic experiments were performed for the human and E. coli systems in which analogous cysteine desulfurase, Fe-S assembly scaffold, and frataxin components were interchanged. Surprisingly, the results have revealed that activation or inhibition by the frataxin homolog is determined by which cysteine desulfurase is present and not by the identity of the frataxin homolog. These data are consistent with a model in which the frataxin-less Fe-S assembly complex exists as a mixture of functional and non-functional states, which are stabilized by binding of frataxin homologs. Intriguingly, this appears to be an unusual example in which modifications to an enzyme throughout evolution invert or reverse the mode of control imparted by a regulatory molecule.

Beneficiary 10 has finally obtained the coordinates of four complexes either involving bacterial frataxin or of competing interactions (IscS/IscU, IscS/CyaY, IscS/Fdx, IscS/YfhJ. The models were deposited in the dada-X database (Hamburg).

Beneficiary 13 used a cellular system combining the expression of mutant human frataxin in a fibroblast cell line completely deleted for endogenous murine frataxin. They demonstrated that the essential function of mammalian frataxin is directly linked to its ability to interact with the ISCU/NFS1/ISD11 complex and that the mature frataxin form hFXN81-210 is the functional form for in vivo Fe-S cluster biosynthesis. Beneficiary 13 has provided evidence that contrary to published data, mammalian frataxin does not interact with ferrochelatase under standard condition. Furthermore, in vivo studies from mouse models previously developed do not support a primary role of frataxin in heme biosynthesis. In parallel, Beneficiary 10 performed a large comprehensive biochemical analysis to determine the potential role of frataxin in heme metabolism, performing binding assays as well as ferrochelatase activities. All the experiments were carried out using human frataxin and human R115L Ferrochelatase in their mature forms. The proteins were purified to homogeneity. The binding of frataxin versus ferrochelatase was first studied by 15N HSQC-NMR. 15N labelled frataxin was titrated with unlabelled ferrochelatase up to 1 to 4 ratios. During the titration no peak perturbation was observed indicating that no binding was taking place between the two proteins. EFACTS data shed strong doubts about previous reports of links between frataxin and ferrochelatase and demonstrates that frataxin (prokaryote and eukaryote) does not interact with ferrochelatase and that it is therefore probably not directly involved in heme biosynthesis.

WP4: Pathogenesis of FRDA

WP4 is dedicated to understanding the pathogenesis pathway involved in FRDA using both model organism and cellular models. This could be accomplished both in large-scale proteomic and transcriptomic analysis, in addition to the exploration of specific pathways that are known to be involved in FRDA.

Beneficiary 12 proposed to define new tools to facilitate the diagnosis and/or monitoring evolving treatments in Friedreich ataxia. They have developed a chronic model of frataxin deficiency by means of RNAi technology, generating stable lines deficient for frataxin in a the human neuroblastoma cell line SH-SY5Y. Neuroblastoma shares a common origin, the neuronal crest, with the glial cells and neurons of the
periphery nervous system, including dorsal root ganglia (DRG). These were used to investigate the role of mitochondria in the pathogenesis of the disease and how the disease affects essential pathways. A second model that Beneficiary 12 has investigated is the dorsal root ganglia from the frataxin deficient mice YG8R (B6.Cg-Fxntm1Mkn Tg(FXN)YG8Pook/J). Using a comparative proteomic profiling study between DRG from YG8R and DRG C57BL/6J mice, 446 proteins have been identified out of 15 differentially expressed spots, all decreased in the YG8R mice versus C57BL/6J animals. The pathway analysis has been performed using the Paintomics visor, a web tool for the integration and visualization of transcriptomics, proteomics and metabolomics data based on the KEGG pathway database. Some of the affected pathways (involving several cellular mechanisms, neuronal processes and metabolic pathways) observed in the proteomic profile have been validated by cellular assays. Mainly electron transport chain and oxidative phosphorylation as well as calcium homeostasis were impaired. This combined cellular and animal models approach of frataxin deficiency reveals common pathogenic mechanisms that leads to pathophysiology of the disease and may define druggable targets.

Beneficiary 13 investigated mitochondrial iron loading and function associated with iron-sulfur cluster deficit seen in Friedreich ataxia. Their results provide key understanding of the fine-tuned regulation of mitochondrial iron needs, by pointing to a specific function of iron regulatory protein 1 (IRP1) in pathological conditions. Activation of IRP1 is responsible for mitochondrial iron accumulation in frataxin deficient mice, and strikingly, deletion of IRP1 revealed that mitochondrial iron metabolism could easily shift from iron overload to iron depletion, subsequently affecting mitochondrial function. In line with the absence of oxidative damage in mitochondria of FxnAlb mice that questions the toxic implication of iron accumulation in the pathophysiology, Beneficiary 13 results therefore suggest that the use of iron chelation as a therapeutic approach in disorders associated with mitochondrial iron accumulation, such as FRDA, should be considered with precaution.

Beneficiary 1 has shown oxidative stress-mediated activation of the intrinsic pathway of apoptosis in β-cells that were rendered frataxin deficient by RNAi. The pro-apoptotic Bcl-2 family members Bad, DP5 and Bim are the key mediators of frataxin deficiency-induced β-cell death. Importantly, the intrinsic pathway of apoptosis is also activated in neurons derived from FRDA patients iPSC (developed in WP6) (Igoillo-Estevé et al., 2015). These neurons have reduced levels of ISC-containing proteins and show signs of oxidative stress. Further findings by Beneficiary 1 suggest a possible interaction between frataxin and the mTOR pathway, a key regulator of cellular metabolism, growth, protein synthesis and autophagy. In iPSC-derived neurons from FRDA patients mTOR activity is low, as indicated by low levels of phosphorylation of its target protein S6, suggesting a state of reduced metabolism and increased autophagy. Interestingly, frataxin upregulation by compounds such as specific HDAC inhibitors restores phospho-S6 levels.

Beneficiary 7 has shown that fibroblasts of FRDA mouse models (YG8R – Beneficiary 9a and KIKO – Beneficiary 1) have a hypersensitivity to oxidation, which can be prevented by reducing lipid peroxidation or activating the Nrf2 pathway. The mitochondrial membrane potential (ΔΨm) was then investigated: Beneficiary 7 found that the two models show a different mitochondrial dysfunction, possibly due to their difference in GAA repeat expansion mutations. YG8R cells showed a depolarization of ΔΨm, which has been found to be a mild impairment in this model while KIKO showed an hyperpolarization of ΔΨm, which could results in a more severe defect. The differences in the length of GAA repeats and the different genetic backgrounds of the two mouse models (one is based on human frataxin and the other on mouse frataxin), may be the cause of the observed mild (YG8R – Beneficiary 9a) and severe (KIKO – Beneficiary 1) mitochondrial bioenergetics defects.
The differences in the two models could in part recapitulate FRDA patient phenotypic variability and therefore be helpful for future drug screening.

WP5: Frataxin Silencing Mechanisms

This WP investigated the molecular mechanism/s whereby the Frataxin (FXN) gene is repressed in FRDA which is central to the pathogenesis of FRDA and is closely linked to WP 8 and 9 where the effect of potential modifiers of epigenotype were examined. Using a variety of different approaches this workpackage has been successful in identifying key principles underlying the mechanisms of how the GAA-repeat expansion represses the Frataxin gene and has developed powerful model systems to both identify potential therapeutic targets aimed at upregulating Frataxin in patients and understand their mechanism of action.

A potentially important finding was that the normally elongating form of RNA polymerase II is stalled within the first exon of the FXN gene; experiments on the dynamics/turnover of this RNAPolII indicated that in normal individuals the RNAPolII is protected from degradation by the proteasome but that in patients with FRDA the proteasome is recruited to the locus and the PolII is rapidly degraded – such a mechanism might contribute to the silencing of the FXN gene (MS under revision). Notably, recent studies suggest that indeed proteasome inhibition can upregulate Frataxin levels albeit by also potentially preventing degradation of the FXN protein itself. Linked to potential mechanisms whereby RNAPolII might be stalled at the FXN gene was the finding that transcribed RNA can hybridise with adjacent DNA, these so-called R-loops were found predominantly in the regions flanking the GAA-repeat itself (Beneficiaries 11, 8 and Dr Natalia Gromak, Oxford). It has been proposed that such structures might play a crucial role in slowing down the rate of transcriptional elongation in FRDA (Groh et al. 2014). Moreover, Beneficiary 11 has developed a cell based model system which allows real-time imaging of transcription of the FXN gene by fluorescently tagging the transcript and performing fluorescence recovery after photobleaching (FRAP) experiments (Silva et al. 2015). These studies show for the first time in living cells, slowing of the FXN transcript on alleles carrying the expanded GAA-repeat and provide a powerful novel tool to investigate how upregulation of FXN might be achieved. Investigation of FXN silencing at the level of individual cells has provided further important insights into the molecular pathology of this disease. Thus, techniques to visualise the nuclear position of the FXN locus in cells have shown that the silenced FXN locus is more likely to associate with the nuclear periphery, this has important implications for understanding how the gene might be maintained in a silent state as this region is thought to represent a silencing compartment in the nucleus and is enriched for particular factors. The techniques developed will allow further studies to determine whether FXN upregulating therapies might act by re-locating the affected locus to the nuclear interior.

Beneficiary 11 has generated FXN genomic DNA-reporter cell models for the rapid quantification of frataxin expression and utilized these models to screen a library of small molecules. This screen identified a compound (C5) which is able to up-regulate FXN mRNA and protein in multiple cell models. This study resulted in a publication on the peer-reviewed journal Human Molecular Genetics (1). Beneficiary 11 has continued improving C5 to ameliorate its properties and has now identified derivatives of C5 which show increased selectivity and potency. This study resulted in the completion of deliverable D6.5. Beneficiary 11 has also developed a FXN genomic DNA-reporter mouse model that provides detectable frataxin expression in live mice, allowing in vivo validation of FXN up-regulating compounds.
Beneficiary9a has developed specific MethylScreen restriction enzyme digestion and qPCR-based protocols to rapidly quantify DNA methylation at four CpG sites in the FXN upstream GAA region (Al-Mahdawi et al. 2013). Increased DNA methylation was confirmed at all four CpG sites in both FRDA cerebellum and heart tissues. Beneficiary9a has also analysed the DNA methylation status in FRDA cerebellum and heart tissues using an approach that enables distinction between 5-hydroxymethylcytosine (5hmC) and 5-methylcytosine (5mC). Analysis revealed that the majority of DNA methylation in both FRDA and unaffected tissues actually comprises 5hmC rather than 5mC. Furthermore, Beneficiary9a identified decreased occupancy of the chromatin insulator protein CTCF (CCCTC-binding factor) at the FXN 5’UTR region in the same FRDA cerebellum tissues. Therefore, increased DNA methylation at the FXN upstream GAA region, primarily 5hmC rather than 5mC, and decreased CTCF occupancy at the FXN 5’UTR are associated with FRDA disease-relevant human tissues. This published data is likely to be important in understanding how the silenced state might become ‘locked-in’ in FRDA.

Importantly, the work from this workpackage had a directly translational aspect contributed to by Beneficiaries 7, 8, 9a. It was shown that the class III histone deacetylase inhibitor, nicotinamide could inhibit heterochromatin at the FXN locus and upregulate expression of FXN (Chan et al., 2013; Libri et al., 2014). This series of experiments were performed initially in cell lines (EBV transformed), primary cells (PBMCs) from patients and a mouse model for FRDA silencing (affected tissues, spinal cord, heart, cerebellum). It was found that nicotinamide treatment rendered the FXN locus more accessible (DNAse I assay), less compacted (3C analysis) with reduced heterochromatin modifications, increased histone acetylation and corrected the expression of previously identified biomarkers (transcriptomic analysis). These findings led to a biochemical proof-of-concept clinical study that established that oral administration of the class III HDACi, nicotinamide, can reduce heterochromatin modifications in primary cells from patients and upregulate the FXN gene in patients (Libri et al., 2014). This exploratory clinical study established that nicotinamide can be safely given at high dose orally to patients and lead to a sustained upregulation of FXN mRNA and protein to those levels found in asymptomatic carriers. The results of this study indicated that a larger and longer randomised placebo controlled clinical trial was warranted to determine clinical efficacy of nicotinamide in FRDA.

As a direct result of their findings Beneficiaries 7, 8 and 11 have secured funding and are now collaborating with Pfizer’s Rare Disease Consortium to further translate their finding towards potential therapies.

WP6: New Disease Models: Cellular & Animals

WP 6 is dedicated to generation of improved mouse and cellular models for FRDA. The specific objectives are 1) to develop improved GAA repeat expansion-based FRDA mouse model, 2) to develop neuronal cell models for FRDA, and 3) to develop iPS cells derived from patients to develop neuronal and cardiomyocytes cellular models, 4) to develop a luciferase based reporter system to study frataxin expression level under different conditions.

EFACTS Beneficiary 9a successfully generated and characterized a mouse model YG8s with longer GAA repeat and a single copy of the transgene. The new mouse model presents a neurological phenotype that is consistent with the known defects in different mouse models. Therefore, this model was useful for further characterization of the phenotype and already enabled to test pharmacological compounds.

In addition, Beneficiary 7 has successfully generated neuronal and glial cell lines derived from the original
EFACTS took advantage of the recent technical advances in the generation of induced pluripotent stem (iPS) cells and their differentiation into various cell types to obtain new cellular models of FRDA from patient’s fibroblasts. This strategy was of particular interest in the case of FRDA as the common mutation is an intronic (GAA)n expansion that shows strong genetic instability, thus making the generation of mouse and cellular models carrying a (GAA)n very challenging. Fibroblasts from two FRDA patients and two healthy controls were reprogrammed to obtain iPS cells by Beneficiary 13 and Beneficiary 1. The obtained iPS cells were validated for all standard criteria. The iPS cells were differentiated into neural progenitors and neurons (Beneficiary 1) as well as active cardiomyocytes (Beneficiary 13). Interestingly, both FRDA iPSC-derived neurons and cardiomyocytes exhibited signs of mitochondrial homeostasis disruption, with decreased mitochondrial potential and progressive mitochondrial degeneration, respectively. These data showed for the first time that FRDA iPSCs and their neuronal and cardiac derivatives represent promising models to study mitochondrial damages and GAA expansion instability in FRDA (Hick et al., 2013).

Beneficiary 11 successfully generated two versions of the FXN-GAA-Luc transgenic mice: FXN-GAA-Luc and FXN-GAA-Luc v2.0. In addition, Beneficiary 11 was successful in constructing a mouse model that demonstrate detectable luciferase expression from the human GAA-expanded FXN locus, allowing studies with focus on detecting luminescence from a live animal. To demonstrate that the FXN-GAA-Luc transgenic mice can be used for in vivo reporter analysis of FXN expression, Beneficiary 11 harvested Cortex, Cerebellum, Heart and Liver tissue from F1 mice and processed to generate lysates and analysed for Luciferase expression. Expression was evident in all tissues compared with just background light emission from a WT control. It’s worth mentioning that technical issues in generating GAA-based mouse models have forced Beneficiaries 8 and 11 to refine their strategies, leading to major delays in this relevant work, but significant results were finally obtained to bring these deliverables to success.

WP7: Development of Biomarkers

Biomarkers are changes in biological parameters that correlate with disease status or disease progression, or changes in response to a given treatment. The identification of biomarkers can provide information on pathogenesis and/or indicate treatment-related changes. A biomarker may correlate to a natural endpoint, such as a change in a measure of neurological impairment. If a direct connection to improved health can be demonstrated, the biomarker can then be used as a surrogate endpoint for evaluating clinical benefit in therapeutic trials. In FRDA, little is available in terms of biomarkers. A few studies have shown changes in markers of oxidative stress in plasma or urine, but results have been variable and difficult to correlate with disease progression or treatment. The overall objective of WP7 is to identify robust markers that can be obtained from relatively simple and minimally invasive procedures, that: 1) correlate with disease status, progression, and/or response to different treatments; 2) can be proposed as surrogate endpoints for clinical studies.

We collected plasma and serum samples, at baseline and after two years, and we initiated a proteome analysis, using a patient cohort comprised of individuals who suffer from early stages of the disease, thus facilitating the comparability with the control group. The proteome analysis was carried out by Beneficiary 2. After testing several methods to deplete the most abundant proteins we have performed a quantitative protein comparison of depleted serum and plasma using age- and sex-matched samples from these two
groups (10 different samples per group). Briefly, pooled samples were proteolytically digested with Trypsin, chemically modified (using stable isotope dimethyl labelling), separated using strong cation exchange (SCX) chromatography and analysed by liquid chromatography/mass spectrometry. We are currently able to quantify 250 proteins in control vs. patient comparisons in both plasma and serum and aim to increase this number to >400 through the use of high performance depletion columns in the near future. For high-resolution mass spectrometry we are using a nanoLC-coupled Orbitrap Elite system. Analysis of the raw mass spectrometry data and protein identification and quantitation is done using the MaxQuant software package with the current human Swissprot database. Initial results show 10 proteins to be differentially regulated. A prominent upregulated protein is alpha-2-macroglobulin, which shows a 1.8-times increase in FRDA patient samples compared to controls. Other proteins showing statistically relevant changes include Apolipoprotein(a) (2-fold upregulated in patients) as well as Apolipoprotein C-II and Apolipoprotein C-III (both 2-fold downregulated in patients). These studies provide the basis for further investigations aimed to validate these markers for use in clinical trials.

Beneficiary 1 has dosed frataxin in PBMCs of EFACTS clinical study patients at baseline and year 2. These data will be used for future clinical trial planning. Beneficiaries 1 and 15 participated in the identification of a gene expression profiles in peripheral blood mononuclear cells (PBMC) that are characteristic of FRDA patients and heterozygous carriers. The FRDA patients’ profile is largely corrected by the ex vivo treatment of PBMCs with drugs that normalize frataxin levels, such as HDAC inhibitors. EFACTS has shown the technical feasibility and reliability of the use of frataxin protein and mRNA levels as biomarkers in clinical studies, including clinical trials. The pursuit of additional peripheral biomarkers, in particular gene expression profiles in PBMCs, has been abandoned and replaced by a search of biomarkers of central nervous system (CNS) physiology. The reasons are 1) that several studies by EFACTS partners and others have already delineated and validated gene expression profile changes in PBMCs of FRDA and defined sets of genes that can be evaluated by qRT-PCR, so additional investigations would add little to the available data; 2) the lack of biomarkers reflecting physiological changes in the CNS, which may play an essential role as outcome measures in future clinical trials if they result to be early indicators of treatment efficacy. Along this line, Beneficiary 1 has started to investigate the functional consequences of the progressive neuronal dysfunction and loss observed in patients with FRDA using Magnetoencephalography (MEG). Sample collection completed for biomarker analysis and cross-sectional correlation with clinical parameters.

WP8: Modifiers of FRDA

The main objectives of WP8 were to establish tractable Drosophila screening models for both FRDA phenotype and GAA repeat-induced gene silencing. The screens would identify conserved pathological pathways and identify key genes in the silencing pathway which could then be interrogated in the mammalian model systems and human samples. These screens have now been validated and several novel pathways identified.

Beneficiary 12 has identified several components of the TOR pathway and some genes involved in metal homeostasis as modifiers of frataxin depletion phenotypes in Drosophila. Their results suggest a metal dysfunction in FRDA including Fe, Zn and probably Cu which implicate the use of chelating agents as potential therapies. Beneficiary12 also found that reduction of TORC1 signalling activity using rapamycin rescues several phenotypes that mimic clinical features of the disease. These results point to TOR
pathway as a novel potential therapeutic target for FRDA. The next challenge will be the identification of molecules targeting the TORC1 outputs regarding oxidative stress defence without affecting other TORC1 signalling pathways. It may provide greater specificity removing undesired side effects of rapamycin (Calap-Quintana et al., 2015).

Beneficiary 12 has performed most of the Drosophila crosses to generate a model capable of screening for genes that will antagonise GAA-mediated silencing. They have tested this system and confirmed that mutation of the classical PEV modifier, Su(var) 2-5 which encodes Heterochromatin protein 1 ameliorates GAA-repeat induced silencing. This finding is consistent with GAA-repeats inducing heterochromatin in Drosophila and provides a platform for further screens to identify other chromatin modifiers likely to be important in the molecular mechanism underpinning GAA-induced gene silencing thereby identifying potential therapeutic targets (MS in preparation). The advantage of the Drosophila system is that it likely to yield potential therapeutic targets that can now be readily tested (by knockout, knockdown or overexpression) in human cell lines (iPS derived neurons or cardiomyocytes) as well as the FRDA mouse model. By analogy with position effect variegation in Drosophila such information is not only likely to provide insight into the establishment and maintenance of silencing in FRDA but also identify novel therapeutic targets.

Beneficiary 9a analysed intergenerational and somatic GAA repeat expansions from YG8 and YG22 FXN transgenic mice that have been crossed with Mlh1 deficient mice. Loss of Mlh1 activity was found to reduce both intergenerational and somatic GAA repeat expansions. However, loss of either Mlh1 or Pms2 also reduced FXN transcription levels, suggesting different mechanisms of action for Mlh1 and Pms2 on GAA repeat expansion dynamics and regulation of FXN transcription. Both MutLα components, PMS2 and MLH1, have now been shown to modify the molecular phenotype of FRDA (Ezzatizadeh et al., 2014). Therefore, upregulation of MLH1 or PMS2 could be potential FRDA therapeutic approaches to increase FXN transcription.

Beneficiary 8 has showed that the PEV modifier HP1gamma binds to the Frataxin gene in patient derived primary cells, EBV cell lines and went on to analyse the effect of knocking down HP1gamma on FXN expression. Although knockdown was effective in the cellular systems there was little effect on FXN expression in FRDA cellular systems. Interestingly, there was a trend towards upregulation of Frataxin in normal cell lines which might implicate HP1gamma in regulation of the normal Frataxin allele. These experiments were complemented by analysing crosses between FRDA model mice from Beneficiary 9a and measuring the effect on Frataxin levels in hemizygous HP1gamma deficient mice in disease relevant tissues. No obvious effect was seen. Following preparation of the 48 month periodic report additional in vivo experiments revealed no effect on Frataxin levels of knocking out a component of the polycomb repressor protein, Bmi 1 which together with the PRC1 complex recognises histone H3K27 trimethylation, a heterochromatin mark found at the FXN locus in FRDA. As there is considerable redundancy in the protein complexes (e.g. HP1alpha, HP1 beta, Mel18) that recognise heterochromatic modifications this might provide an explanation for these results. The heterochromatin at the Frataxin locus in FRDA is somewhat unusual in this respect as both H3K9me3 and H3K27me3 are present in the disease state. Following on from the Drosophila screens it will be important in the future to examine the effect of the modifiers identified in mammalian systems.

WP9: Development & Testing of Therapeutic Strategies

WP9 has the following objectives: 1) drug discovery; 2) studies on mechanisms of action of candidate
This WP has interactions with all other WPs, as it is at the core of the translational research process. These interactions are reciprocal, with clinical studies and the implementation of the patients’ database and IT platform (WP1 and 2), studies on frataxin function (WP3), pathogenesis (WP4), epigenetics (WP5), biomarkers (WP7) and modifiers (WP8) feeding into the discovery and development of new treatment for FRDA and in turn receiving critical inputs from studies on new therapeutics.

Beneficiary 11 has identified RNA/DNA hybrids (R-loops) as a common feature of nucleotide expansion disorders including FRDA, providing a new target for therapeutic interventions (Groh et al., 2014). They showed that R-loops form in patient cells on expanded repeats of endogenous FXN and FMR1 genes, associated with FRDA and FXS. These transcription-dependent R-loops are stable, co-localize with repressive H3K9me2 chromatin mark and impede RNA Polymerase II transcription in patient cells. Importantly, increasing R-loop levels by treatment with DNA topoisomerase inhibitor camptothecin leads to up-regulation of repressive chromatin marks, resulting in FXN transcriptional silencing. This provides a direct molecular link between R-loops and the pathology of TREDs, suggesting that R-loops act as an initial trigger to promote FXN and FMR1 silencing.

Beneficiary 13, working on the development of AAV-based gene therapy for FRDA, obtained very promising results using an AAV9 vector expressing human frataxin in early symptomatic treatment in a neuronal conditional mouse model (FXN L3/L-; Pvalbcrc+). In particular, a complete prevention of loss of sensorimotor reflexes is found. It is now important to validate a proof-of-concept that gene therapy could be beneficial at an advanced stage of the disease, as FRDA patient already show symptoms at the time of diagnosis. These studies are the basis for future developments after the present FP7 project.

After Beneficiaries 1 and 15 further elucidated the mechanism of action of the 2-aminobenzamides HDAC inhibitors (Soragni et al., 2014) Beneficiary 11 provided further information on mechanisms of gene silencing at the FXN locus and the mechanism of action of HDAC inhibitors. They demonstrated inefficiencies in transcription initiation and elongation from the expanded GAA-FXN locus at single-cell resolution. By visualizing FXN expression and nuclear localization in single cells, they showed that GAA-expanded repeats decrease the number of FXN mRNA molecules, slow transcription, and increase FXN localization at the nuclear lamina (NL). Importantly for therapeutic applications, restoring histone acetylation increases FXN transcription and reverses NL positioning (Silva et al., 2015).

Beneficiary 1 has undertaken testing of newer aminobenzamide HDAC inhibitors in iPSC-derived neurons and in a mouse model (the KIKI mouse). The development of these molecules has been described in the previous report. Briefly, compared to the compound tested by Beneficiaries 1 and 15 in a pilot clinical trial (RG2833) (Soragni et al., 2014), these newer compounds have much improved drug properties, including better penetration in the central nervous system, longer half-life and no production of potentially toxic metabolites as it was the case for RG2833 (a long-lived potentially cardiotoxic benzimidazole and products of amide hydrolysis, including o-phenylenediamine (OPD), acetylated OPD (AcOPD) and acids). Preliminary results indicate so far that they can strongly induce the expression of frataxin in iPSC-derived neurons.

Beneficiary 8, after the successful completion of a pilot study (Libri et al., 2014) is planning a phase 2 trial with the Class II HDAC inhibitor nicotinamide.

Very importantly, the continuation of the EFACETS clinical study, involving Beneficiaries 1, 2, 5, 6, 7, 14 and 16 and the ongoing analysis of its results are providing critical information for future trial design.
Overall EFACTS could achieve its translational goals by providing a better understanding of FRDA through basic research tools and by identifying novel disease-specific biomarkers and drug therapeutics. This was coupled to improving the methods of clinical assessment and diagnosis, and the implementation of the first pan-European FRDA database registry, linked to bio banks of patient material.

References


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Potential Impact:
Friedreich ataxia is a rare neurological disease with a prevalence in the order of 1 per 30,000 in the European population. There is no pharmacological treatment currently available for the disease, but an advantage is that we know the primary disease-causing gene - frataxin - associated with the disease. The EFACTS consortium have amassed a Europe-wide body of expertise, spanning the spectrum of research from basic mechanisms to clinical care, to create critical mass that acted as an impetus in pushing forward our understanding of the clinical progression and mechanisms of disease and provide new therapeutic solutions. As FRDA is a rare disease, the EFACTS strategy has adopted a 3-pronged approach to its research to bring maximal impact:
• Clinical research - its actions have shed light on the course of the disease and facilitated design of future clinical trials.
• Mechanistic research - had a major impact on our understanding of the molecular bases contributing to FRDA etiology.
• Therapeutic research – identified, developed and tested therapeutics on FRDA models and in early
Clinical research. The results of the cross sectional baseline analysis of the EFACTS cohort (~600 patients) suggest that earlier disease onset is associated with larger numbers of GAA repeats and more rapid disease progression. The differential estimated progression of ataxia symptoms related to age of onset have implications for the design of clinical trials in Friedreich ataxia, for which SARA might be the most suitable measure to monitor disease progression. The continuation of the EFACTS clinical study has started to provide prospective data. Preliminary analyses of the 1-year follow-up data confirm the value of SARA as outcome measure of clinical progression, and indicate the ADL scale as another robust progression marker directly related to the patients’ functioning in everyday life. Among performance tests, the CCFS, which EFACTS validated in children as well (Filipovic Pierucci et al., 2015) emerged as the most sensitive and robust. We expect that the analyses of the 2- and 3-year follow-up data will further delineate a set of reliable clinical outcome measures, allowing power calculations for clinical trials and rational trial design.

Along with the clinical assessment tools, EFACTS has been developing biomarkers reflecting disease status, progression, and response to therapeutics. The original focus has been on biochemical markers, with the more recent addition of neurophysiological markers. The combination of genetics, functional and cognitive data, and biomaterial samples in EFACTS registry not only provides reliable predictors of disease progression for clinical trials, it also contributes to our understanding of disease pathophysiology and identification of novel therapeutic targets.

In addition, the EFACTS network of clinical sites by itself provides an essential infrastructure for future clinical trials in Europe, combining medical expertise and patient availability. The patients’ registry/database in turn allows rapid and effective recruitment of patients for trials. It is therefore not surprising that EFACTS has started fruitful interactions with companies involved in the development of therapeutics for FRDA. These interactions involve collaboration for data analysis, definition of outcome measures and overall trial design.

Another impact of the EFACTS clinical studies has been improved knowledge of the disease and its impact of patients’ lives. One way EFACTS investigators have put to fruition this knowledge has been their participation to the development of detailed consensus clinical management guidelines. Together with colleagues located in Australia, Canada and USA, EFACTS clinicians used the experience gained while carrying out the project to critically appraise published evidence related to FRDA clinical care and generate recommendations. Where no published data specific to FRDA existed, recommendations were based on data related to similar conditions and/or expert consensus. There were 146 recommendations developed to ensure best practice in the delivery of health services to people with FRDA. A succinct summary of these guidelines has been published in a medical journal (Corben et al., 2014) and the full guidelines are available in the web sites of patients’ organizations. Whilst the development of these guidelines provides a critical first step in the provision of appropriate clinical care for people with FRDA, it also highlights the urgency of continuing and further expanding high-quality clinical studies such as the EFACTS clinical project, that will ensure the delivery of optimum clinical management and intervention for people with FRDA.

Overall, the EFACTS clinical project has impacted the medical field by raising awareness of this and other rare neurological diseases, providing wider access to expert care and to tools for how best to diagnose and manage the disease, and putting the basis for effective design and implementation of clinical therapeutic investigations.

Mechanistic research. EFACTS investigators have provided major contributions to the substantial
progress in understanding FRDA pathogenesis that has occurred since the start of the project in 2010. Studies on frataxin function have cleared the field of inconsistent hypotheses while clarifying frataxin’s essential role in ISC biogenesis. EFACTS investigators have provided key data on frataxin’s physical and functional interactions in the ISC biogenesis complex in the mitochondrial matrix, up to fine structural details.

Defective ISC synthesis has been recognized as the primary cause leading to cellular dysfunction and death due to frataxin deficiency. At the same time, essential details have emerged on the chain of events occurring in different types of frataxin-deficient cells, leading to the identification of novel pathways involved in FRDA pathogenesis. Very importantly, these studies on the one hand indicate novel potential therapeutic targets, and on the other end reveal limits and liabilities of previously proposed treatment approaches.

At a more basic level, EFACTS investigators have contributed to dissect the epigenetic mechanisms underlying FXN gene silencing by the expanded GAA repeats, guiding therapeutic interventions targeting the disease at its very roots.

Finally, mechanistic studies were linked to clinical studies, particularly in the identification and evaluation of biomarkers, and interpretation of biomarker data.

Therapeutic research. Therapeutic research has been seen since the implementation of EFACTS as the point of convergence of all its components. Remarkable progress has been made not only on the identification of potential therapeutics, but also in the their pre-clinical and clinical development. Two early clinical trials, one with nicotinamide and one with the benzamide HDAC inhibitor RG2833, have involved EFACTS investigators and were based on data partly generated by EFACTS investigators.

Overall impact. EFACTS partners confirmed to be undisputed leaders in FRDA research. Major contributions of the project include progress in understanding the genetic and epigenetic basis of the disease, the function of frataxin, the generation of iPS cell-derived cardiac and neuronal cell models and of novel mouse models for the disease, the creation of a European patient registry, the definition of a panel of clinical assessment tools to be used in clinical trials, the identification of several possible therapeutic approaches and the active participation in several clinical trials.

Thanks to EFACTS dissemination activities, we improved awareness of FRDA, made an impact on the QOL of patients and families, provided updated information on the disease, on standards of care, on treatment options to lay and professional organizations and facilitated access to specialized centres.

The development of therapies for FRDA cannot happen without industrial involvement. EFACTS has included an industrial partner from the beginning and has expanded its collaboration with companies, both for clinical trial design and planning, and for pre-clinical development of therapies for FRDA.

Links with patient advocacy group are of particular importance. While all EFACTS partners have long-standing links with the respective national patient advocacy groups, we launched an initiative to partner with Euro-ataxia, the not-for-profit federation of 16 national ataxia patient groups from across Europe. This partnership is the best assurance that our efforts will continue to improve the life of the end users of this research, FRDA sufferers.

